

Abstracts

Biochemistry and nutrition

ON THE PHYSIOLOGICAL BASIS OF VITAMIN A-STIMULATED GROWTH. M.H. Zile, E.C. Bunge and H.F. DeLuca (Dept. of Biochem., College of Agricultural and Life Sciences, Univ. of Wisconsin, Madison, Wis. 53706) *J. Nutr.* 109(10), 1787-96 (1979). The influence of vitamin A depletion on tissue composition was studied in rats that were marginally vitamin A deficient, i.e. at their weight plateau stage. The total number of cells (DNA) was decreased in most organs as a result of vitamin A depletion. In thymus, spleen and the sublingual glands there was also a dramatic reduction in the number of cells per gram of tissue and in thymus and sublingual glands there was an increase in the protein to DNA ratio as a result of absence of dietary vitamin A. We present the hypothesis that vitamin A stimulates growth by a direct role in cell replication in addition to or instead of stimulating the differentiation of epithelial and bone cells.

STRUCTURE OF THE MAJOR GANGLIOSIDES FROM BOVINE THYROID. G.A.F. Van Dessel, A.R. Lagrou, H.J.J. Hilderson, W.S.H. Dierick, and W.F.J. Lauwers. (UIA-Laboratory for Pathological Biochem. and RUCA-Laboratory for Human Biochem., Univ. of Antwerp, Groenenborgerlaan 171, B2020 Antwerp, Belgium) *J. Biol. Chem.* 254(18), 9305-10 (1979). After preparative isolation, the carbohydrate, long chain base, and fatty acid composition of the major gangliosides from bovine thyroid have been analyzed. The structures were elucidated by determining the molar ratio of the building blocks, permethylation analysis, and enzymatic degradation studies. The following structures are identified: N-Acetylneuraminyl(2,3)-galactosyl(1,4)glucosyl(1,1)ceramide; N-glycolylneuraminyl(2,3)galactosyl(1,4)glucosyl(1,1)ceramide; galactosyl(1,3)N-acetylgalactosaminyl[(3,2)N-acetylneuraminyl](1,4)galactosyl(1,4)glucosyl(1,1)ceramide; fucosyl(1,2)galactosyl(1,3)N-acetylgalactosaminyl[(3,2)N-acetylneuraminyl](1,4)galactosyl(1,4)glucosyl(1,1)-ceramide. The structures were confirmed by direct inlet mass spectrometry of the permethylated gangliosides and the corresponding sialo derivatives. Structures are proposed for common ions in the different mass spectra.

CYCLOPROPANE FATTY ACID SYNTHASE OF *ESCHERICHIA COLI*. STABILIZATION, PURIFICATION, AND INTERACTION WITH PHOSPHOLIPID VESICLES. F.R. Taylor and J.E. Cronan (Dept. of Molecular Biophy. and Biochem., Yale Univ., New Haven, Connecticut) *Biochemistry* 18(15), 3292-300 (1979). The cyclopropane fatty acid (CFA) synthase of *Escherichia coli* catalyzes the methylation of the unsaturated moieties of phospholipids in a phospholipid bilayer. The methylene donor is S-adenosyl-L-methionine. The enzyme is loosely associated with the inner membrane of the bacterium and binds to and is stabilized by phospholipid vesicles. The enzyme has been purified over 500-fold by flotation with phospholipid vesicles and appears to be a monomeric protein having a molecular weight of about 90,000. The enzyme binds only to vesicles of phospholipids which contain either unsaturated or cyclopropane fatty acid moieties. Studies with a reagent that reacts only with the phosphatidylethanolamine molecules of the outer leaflet of a phospholipid bilayer indicate the CFA synthase reacts with phosphatidylethanolamine molecules of both the outer and the inner leaflets of phospholipid vesicles.

ASYNCHRONOUS APPEARANCE OF TWO ENZYMES CONCERNED WITH MEDIUM CHAIN FATTY ACID SYNTHESIS IN DEVELOPING RAT MAMMARY GLAND. S. Smith and P. Ryan (Bruce Lyon Memorial Research Lab., Children's Hospital Medical Center of Northern California, Oakland, CA 94609) *J. Biol. Chem.* 254(18), 8932-6 (1979). The time course for development of the capacity for medium chain (C₆ to C₁₂) fatty acid synthesis, by rat mammary gland, has been examined and compared with changes in the levels of fatty acid synthetase and thioesterase II. The latter enzyme regulates the production of medium chain fatty acids in mammary gland by hydrolyzing medium chain acyl moieties from thioester linkage to the fatty acid synthetase. At all stages of development, the level of thioesterase II present in the mammary gland correlates closely with the potential for medium chain fatty acid synthesis, whereas the level of fatty acid synthetase correlates closely with overall lipogenic capacity. The significance of the asynchronous development of these two key enzymes during mammary gland differentiation is discussed.

CLINICAL EXPERIENCE WITH THE SOYBEAN PROTEIN DIET IN THE TREATMENT OF HYPERCHOLESTEROLEMIA. C.R.

Sirtori, E. Gatti, O. Mantero, F. Conti, E. Agradi, E. Tremoli, M. Sirtori, L. Fraterrigo, L. Tavazzi, and D. Kritchevsky (Dept. of Nutrition, Maggiore Hospital, Milano, Italy) *Am. J. Clin. Nutr.* 32(8), 1645-58 (1979). The efficacy of the total substitution of animal protein with a textured soybean protein in hypercholesterolemic individuals was assayed in 42 in-patients and 18 out-patients. The in-patients studied followed one of three different crossover protocols: in protocol A, the soybean diet was compared with a standard low lipid diet; protocol B compared two soybean diets, one with added cholesterol, one without; and protocol C compared a soybean diet containing a high P/S fatty acid ratio to one with a low P/S ratio. In all three protocols, the soybean regimen provided valid and reproducible hypocholesterolemic effects that were not modified by the addition of cholesterol. P/S variations appeared, however, to modify the final effect: soybean definitely had a decreased effectiveness with a low P/S (0.1) regimen. These studies indicate that treatment with the soybean diet is an effective regimen for inducing a significant cholesterol reduction in type II patients refractory to standard low lipid regimens.

α HYDROXYLATION OF LIGNOCERIC ACID IN BRAIN. SUBCELLULAR LOCALIZATION OF α HYDROXYLATION AND THE REQUIREMENT FOR HEAT-STABLE AND HEAT-LABILE FACTORS AND SPHINGOSINE. I. Singh and Y. Kishimoto. (John F. Kennedy Institute for Handicapped Children and Dept. of Neurology, Johns Hopkins Univ. School of Medicine, Baltimore, MD 21205) *J. Biol. Chem.* 254(16), 7698-704 (1979). The conversion by α hydroxylation of lignoceric acid to cerebronic acid required a heavy particulate fraction prepared by KCl-Mg²⁺ differential centrifugation and two cytoplasmic factors. This α -hydroxylase activity varied linearly when the concentrations of the heat-stable (HSF) and heat-labile (HLF) factors were changed. On the other hand, the α -hydroxylase activity showed an initial lag and then increased linearly when the amount of particulate fraction was varied. All of the newly synthesized cerebronic acid was found as a constituent of ceramide and cerebroside, and the α hydroxylation of lignoceric acid and lignoceroyl-CoA was stimulated by the addition of sphingosine. In this system 3-³H sphingosine was incorporated into hydroxyceramide and hydroxycerebroside. These observations indicate that α hydroxylation is closely integrated with the synthesis of sphingosine, ceramide, and cerebroside. The ratios of 1-¹⁴C lignoceric acid to the 3-³H sphingosine incorporated in hydroxyceramide and hydroxycerebroside were identical; this indicates that all hydroxycerebroside was made from hydroxyceramide.

THE EFFECTS OF CHOLESTYRAMINE ON HIGH DENSITY LIPOPROTEIN METABOLISM. J. Shepherd, C.J. Packard, J.G. Morgan, J.L.H.C. Third, J.M. Stewart and T.D. Veitch Lawrie. (Univ. Dept. of Biochemistry and Univ. Dept. of Medical Cardiology, Royal Infirmary, Glasgow G4 0SF, Great Britain) *Atherosclerosis* 33(4), 433-44 (1979). This study on 4 type II hyperlipoproteinaemic subjects examines the effects of pharmacologic doses (8g twice daily) of the bile acid sequestrant cholestyramine on the plasma distribution and chemical composition of the high density lipoprotein subfractions, HDL₂ and HDL₃, and describes the influence of the drug on the metabolism of the major HDL apoproteins, apolipoprotein A-I and A-II. Cholestyramine lowered plasma low density lipoprotein cholesterol (32%; P<0.05) without affecting the level of that lipid in very low density or high density lipoproteins. However, the plasma HDL₂/HDL₃ ratio and apolipoprotein A-I concentration rose significantly on treatment, while apolipoprotein A-II remained unchanged. The rise in apolipoprotein A-I derived from an increase in its synthetic rate and produced a relative enrichment of the protein with respect to apolipoprotein A-II in both HDL subfractions. These results demonstrate the cholestyramine treatment affects HDL metabolism in a way which, according to current concepts, may prove beneficial to the recipient.

VITAMIN K-DEPENDENT CARBOXYLASE: LIVER ACTIVITY IN VARIOUS SPECIES. D.V. Shah and J.W. Suttie (Dept. of Biochem., Univ. of Wisconsin, Madison, WI 53706) *Proc. Soc. Exp. Biol. Med.* 161(4), 498-501 (1979). Liver microsomes of eight species (Holtzman- and Warfarin-resistant rats, mice, guinea pigs, hamsters, rabbits, calves, pigs, and chickens) have been assayed for the vitamin K-dependent carboxylase activity that converts protein-bound glutamyl residues to γ -carboxyglutamyl residues. Development of hypoprothrombinemia by anticoagulant treatment or vitamin K deficiency caused a two- to fourfold increase in liver carboxylase activity in all species except in the guinea pig. The carboxylase activity in the hamster was about three-fold higher than rat than that of mice, rabbit and pig. Microsomes from chicken or

calf liver had very low vitamin K-dependent peptide carboxylase activity.

THE EFFECT OF β -PYRIDYLCARBINOL ON LIPOPROTEIN LIPIDS IN PRIMARY TYPE IIA HYPERLIPOPROTEINEMIA. R. Schwandt, P. Weisweiler and G. Neureuther (2nd Medical Clinic, Klinikum Grosshadern, Univ. of Munich, Marchionistr. 15, D-8000 Munich 70, F.R.G.) *Atherosclerosis* 34(1), 35-9 (1979). The effect of long-term treatment over 6 months with β -pyridylcarbinol on the lipoprotein lipids was investigated in 12 patients with primary type IIA hyperlipoproteinemia. VLDL, LDL, and HDL were separated by preparative ultracentrifugation. There was a significant decrease of serum cholesterol and phospholipids. The normal serum triglycerides were unaffected. While VLDL and HDL lipids showed no significant alterations, the LDL lipids decreased. The atypical lipid composition of the LDL was changed towards normal. Though there was a significant decrease of the "atherogenic" LDL/HDL-lipid ratios after 6 months treatment, β -pyridylcarbinol did not result in a normalization of this ratio.

HYDROLYSIS OF MEMBRANE PHOSPHOLIPIDS BY PHOSPHOLIPASES OF RAT LIVER LYSOSOMES. D.E. Richards, R.F. Irvine and R.M.C. Dawson (Dept. of Biochem., A.R.C. Inst. of Animal Physiology, Babraham, Cambridge CB2 4AT, U.K.) *Biochem. J.* 182(2), 599-606 (1979). The hydrolysis of 32 P- or myo-[2- 3 H]inositol-labelled rat liver microsomal phospholipids by rat liver lysosomal enzymes has been studied. The relative rates of hydrolysis of phospholipids at pH 4.5 are: sphingomyelin > phosphatidylethanolamine > phosphatidylcholine > phosphatidylinositol. The predominant products of phosphatidylcholine and phosphatidylethanolamine hydrolysis are their corresponding lyso-compounds, indicating a slow rate of total deacylation. Comparisons are drawn between the hydrolysis by lysosomal enzymes of membrane substrates and that of pure phospholipid substrates, and also the possible role of phospholipid substrates, and also the possible role of phosphatidylinositol-specific lysosomal phospholipase C in cellular phosphatidylinositol catabolism is discussed.

SYNTHESIS AND CONTENT OF ETHER-LINKED GLYCEROPHOSPHOLIPIDS IN THE HARDERIAN GLAND OF RABBITS. A. Radomska-pyrek, A. Dabrowiecki and L.A. Horrocks (Dept. of Physio. Chem., The Ohio State University, 1645 Neil Avenue, Columbus, OH 43210) *Biochim. Biophys. Acta* 574(2), 248-57 (1979). Although harderian glands are rich in neutral glycerolipids with ether bonds, less than 20% of the choline glycerophospholipids have ether bonds in the white and pink portions of the adult rabbit harderian gland. Only 6% of these are plasmalogens while 94% are alkylacyl glycerophosphocholines. The ethanolamine glycerophospholipids include 37% with ether bonds in both white and pink portions. In the white portion 96% are plasmalogens but only 19% are plasmalogens in the pink portion. The microsomal ethanolamine-phosphotransferase (EC 2.7.8.1) is more active with diacylglycerols than with alkylacylglycerols. The microsomal cholinephosphotransferase (EC 2.7.8.2) is equally active with both diradylglycerols. Particularly with microsomes from the pink portion, the apparent K_m values for CDPethanolamine and CDPcholine are lower in the presence of alkylacylglycerols than in the presence of diacylglycerols. The incorporation of radioactivity from CDP[14 C] ethanolamine and CDP[14 C] choline into ethanolamine and choline plasmalogens was increased several-fold by addition of alkylacylglycerols but was not increased substantially by addition of diacylglycerols.

INHIBITION OF FATTY ACID OXIDATION BY 2-BROMO-OCTANOATE. EVIDENCE FOR THE ENZYMATIC FORMATION OF 2-BROMO-3-KETO-OCTANOYL COENZYME A AND THE INHIBITION OF 3-KETOTHIOLASE. B.M. Raaka and J.M. Lowenstein (Grad. Dept. of Biochem., Brandeis Univ., Waltham, Mass. 02154) *J. Biol. Chem.* 254(14), 6755-62 (1979). Incubation of rat liver mitochondria with 10 μ M DL-2-bromooctanoate causes complete and irreversible inactivation of 3-ketothiolase (acyl-CoA:acetyl-CoA C-acyltransferase). Evidence is presented that mitochondria convert bromooctanoate to 2-bromo-3-keto-octanoyl-CoA, an α -haloketone which is probably the active form of the inhibitor. The inactivation is accompanied by incorporation of radioactivity from [1- 14 C] bromooctanoate into the enzyme. Bromooctanoate does not affect the activities of the other enzymes of β -oxidation, except for 3-ketothiolase II (acetyl-CoA:acetyl-CoA C-acetyltransferase), which becomes partially inhibited. Evidence is also presented that various enzymes of β -oxidation can use 2-bromooctanoyl-CoA and its β -oxidation products as substrates.

PARTIAL AND INCOMPLETE OXIDATION OF PALMITATE BY CULTURED BEATING CARDIAC CELLS FROM NEONATAL RATS. A. Pinson, J. Desgres, and M. Heller (Myocardial Res. Group, Dept. of Biochem., Hebrew Univ.-Hadassah Med. School, Jerusalem, Israel) *J. Biol. Chem.* 254(17), 8331-5 (1979). The discrepancy in

the rate of [14 C]O₂ formation from either [1- 14 C]- or [16- 14 C] palmitate is demonstrated and could be explained by the preferential formation of L-(+)-3-hydroxybutyrate from the four carbon atoms at the ω terminus. The identity of this product as L-(+)-3-hydroxybutyrate was established and shown to be the major component of the radioactive products in the extracellular medium from palmitate based on (a) ion-exchange chromatographical properties, (b) gas-liquid chromatography, (c) mass spectrometric analysis, (d) stereoisomeric separation, and (e) its very low rate of utilization by the cells. We therefore propose a shunt to the oxidation of palmitate in these cells occurring at the stage of L-(+)-hydroxybutyryl-CoA which undergoes deacylation causing the product to be transported outside the cell.

PORTACAVAL SHUNT AND WHOLE-BODY CHOLESTEROL METABOLISM IN THE CHOLESTEROL-FED AFRICAN GREEN MONKEY. J.S. Parks, N.D.M. Lehner, R.W. St. Clair, and H.B. Lofland (Bowman Gray Sch. of Med. of Wake Forest Univ., Winston-Salem, NC 27103) *Proc. Soc. Exp. Biol. Med.* 161(4), 502-7 (1979). African green monkeys consuming a diet containing 0.62 mg cholesterol/Cal and 40% of calories as fat were characterized for whole-body cholesterol metabolism after control (sham) or portacaval shunt operations. Whole-body cholesterol metabolism was studied by three different methods: sterol balance analysis, serum cholesterol turnover analysis, and feeding radiolabeled cholesterol until a serum cholesterol isotopic steady state was reached. We found no difference between control and portacaval shunt animals for the following characteristics: serum cholesterol concentration, excretion of neutral steroids or bile acids, cholesterol absorption or synthesis, and kinetic parameters of serum cholesterol turnover. We have concluded that portacaval shunt in African green monkeys fed a diet containing high fat and a moderate amount of cholesterol had no effect on whole-body cholesterol metabolism.

PROPERTIES OF SALT-RESISTANT LIPASE AND LIPOPROTEIN LIPASE PURIFIED FROM HUMAN POST-HEPARIN PLASMA. A.-M. Östlund-Lindqvist (Dept. of Med. and Physio. Chem., Biomedical Centre, Univ. of Uppsala, Uppsala, Sweden) *Biochem. J.* 179(3), 555-9 (1979). Lipoprotein lipase and salt-resistant lipase were isolated from human post-heparin plasma. The proteins of human post-heparin-plasma lipoprotein lipase and salt-resistant lipase were identified and demonstrated to be immunologically different. Significant differences between the two enzymes in their relative amino acid composition were demonstrated, which indicates that the two enzymes are different proteins. When analysed by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis, the enzymes seemed to have monomeric weights similar to that of lipoprotein lipase purified from bovine milk.

EFFECT OF PRE- AND POSTNATAL ESSENTIAL FATTY ACID DEFICIENCY OF BRAIN DEVELOPMENT AND MYELINATION. M.C. McKenna and A.T. Campagnoni (Dept. of Chemistry, Univ. of Maryland, College Park, Maryland 20742) *J. Nutr.* 109(7), 1195-204 (1979). Pregnant mice were fed an essential fatty acid (EFA) deficient diet from day 1 of gestation. Several biochemical parameters of postnatal brain growth and myelination were measured on their progeny and compared with controls fed a normal diet containing 4% corn oil or a commercial breeder diet. Measurements of brain DNA, RNA and protein content of the EFA deficient mice suggested a retardation of brain growth and development of about 1 week compared to controls, with the most striking differences noted at ages below 15 days. DNA content of both control and experimental mice became comparable at 20 to 22 days but brain protein and RNA content remained lower in deficient mice at all ages studied. The results indicate that pre- and postnatal EFA deficiency results in a retardation of brain development and a profound reduction of some but not all myelin specific components.

THE EFFECT OF A NON-ABSORBABLE FAT, SUCROSE POLYESTER, ON THE METABOLISM OF VITAMIN A BY THE RAT. F.H. Mattson, E.J. Hollenback and C.M. Kuehlthau (Proctor & Gamble Co., Miami Valley Laboratories, P.O. Box 39175, Cincinnati, OH 45247) *J. Nutr.* 109(10), 1688-93 (1979). Sucrose polyester (SPE) is a fat-like material that is not absorbed. The effect of this material on vitamin A metabolism was determined by measuring the amount of the vitamin that was stored in the liver of rats following the ingestion of a known amount of vitamin A. In one study, the vitamin A was administered as an oral dose in a vehicle consisting of various proportions of cottonseed oil and SPE. Each 1% replacement of cottonseed oil by SPE resulted in a 0.26% decrease in the amount of vitamin A found in the liver. In the second study, the vitamin A was incorporated into diets in which the fat component consisted of various proportions of cottonseed oil and SPE. When these diets were consumed for 1 week, each 1% replacement of cottonseed oil by SPE resulted in a 0.84% decrease in the storage of vitamin A by the liver. It is proposed that in the lumen of the intestine vitamin A distributes between the customary micellar

phase and the unhydrolyzed oil phase of SPE. The vitamin A in this latter phase is eliminated in the feces.

EXCRETION OF SOAP FATTY ACIDS BY CHICKS FED DIETS OF DIFFERENT COMPOSITION. B.E. March and C. MacMillan (Dept. of Poultry Science, Univ. of British Columbia, Vancouver, Canada V6T 1W5) *Poult. Sci.* 58(5), 1246-9 (1979). White Leghorn chicks were fed diets containing either soybean meal or rapeseed meal as the source of supplementary protein. The diets were modified by the addition of corn oil, corn oil plus erucic acid, cornstarch, or herring meal. Excreta from the chicks were analyzed for ether-soluble lipid and for the amounts of fatty acids present in soaps. The birds fed the different soybean meal-containing diets in three experiments excreted, on the average, 30.2% of lipid in the form of soap fatty acid. Those fed the equivalent diets containing rapeseed meal excreted 16.6% of lipid in the form of soap fatty acid. Differences in the proportions of fatty acids excreted as soaps are attributed primarily to differences in concentration of ionic calcium in the intestinal lumen.

MITOCHONDRIAL AND PEROXISOMAL FATTY ACID OXIDATION IN LIVER HOMOGENATES AND ISOLATED HEPATOCYTES FROM CONTROL AND CLOFIBRATE-TREATED RATS. G.P. Mannaerts, L.J. Debeer, J. Thomas, and P.J. De Schepper (Afdeling Farmakologie, Faculteit Geneeskunde, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium) *J. Biol. Chem.* 254(11), 4585-95 (1979). Mitochondrial and peroxisomal fatty acid oxidation were compared in whole liver homogenates. Oxidation of 0.2mM palmitoyl-CoA or oleate by mitochondria increased rapidly with increasing molar substrate:albumin ratios and became saturated at ratios below 3, while peroxisomal oxidation increased more slowly and continued to rise to reach maximal activity in the absence of albumin. Under the latter condition mitochondrial oxidation was severely depressed. In homogenates from normal liver peroxisomal was lower than mitochondrial oxidation at all ratios tested except when albumin was absent. Assuming that all H₂O₂ produced during fatty acid oxidation was due to peroxisomal oxidation, it was calculated that the contribution of the peroxisomes to fatty acid oxidation was less than 10% both in cells from control and clofibrate-treated animals.

PLASMA LIPID DISTRIBUTIONS IN SELECTED NORTH AMERICAN POPULATIONS: THE LIPID RESEARCH CLINICS PROGRAM PREVALENCE STUDY. The Lipid Res. Clinics Prog. Epidemiology Committee. (Lipid Metabolism Branch, NHLBI, NIH Bethesda, Maryland 20205) *Circulation* 60(2), 427-39 (1979). Cross-sectional age- and sex-specific plasma lipid distributions (means, medians and selected percentiles) are given for 48,431 white participants in visit 1 of the Lipid Research Clinics (LRC) Prevalence Study. These data confirm findings from earlier studies in developed countries, showing age-related differences plasma lipid levels. However, for overall distributions, the LRC data showed slightly lower cholesterol and markedly higher triglyceride values than those previously reported for North America. The large number of participants within most subgroups permitted a variety of analytic and comparative studies. For example, data from the large pediatric population revealed a drop in plasma cholesterol levels in adolescent males and females. Males aged 20-50 years had higher cholesterol levels than females in the same age group, and higher triglyceride levels between ages 20-70 years. Numbers were also sufficient for meaningful comparisons between lipid distributions of females who were taking sex hormones and those who were not: In females taking sex hormones, cholesterol and triglyceride levels were higher for subjects younger than 45 years, but slightly lower after age 45, than lipid levels in females not taking hormones.

MAINTENANCE ENERGY REQUIREMENTS, ENERGY RETENTION AND HEAT PRODUCTION OF YOUNG OBESE (OB/OB) AND LEAN MICE FED A HIGH-FAT OR A HIGH-CARBOHYDRATE DIET. P.Y. Lin, D.R. Romsos, J.G. Vander Tuig and G.A. Leveille (Dept. of Food Science and Human Nutrition, Michigan State Univ., East Lansing, MI 48824) *J. Nutr.* 109(7), 1143-53 (1979). Female obese (ob/ob) and lean mice were weaned at 21 days of age, placed in wire-meshed cages maintained at 25 to 30°, and fed a high-fat or a high-carbohydrate diet for 21 days. The body energy balance procedure was utilized to determine the maintenance energy requirements, and the efficiency of dietary energy utilization, above maintenance, in these mice. Heat production, per unit body weight, was lower in obese mice than in lean mice. The lowest heat production was observed in obese mice fed the high-fat diet. The 40% lower maintenance energy requirement of the obese mice is a major factor contributing to the high efficiency of energy retention in these mice. Consumption of a high-fat diet further improved the ability of the obese mice to retain dietary energy.

SELECTIVE MEASUREMENT OF TRIACYLGLYCEROL LIPASE

ACTIVITIES IN PIG POST-HEPARIN PLASMA. T. Kuusi, T. Schröder, B. Bang, M. Lempinen and C. Ehnholm (Third Dept. of Med. and Second Dept. of Surgery, Univ. of Helsinki and Central Public Health Lab., Helsinki (Finland)). *Biochim. Biophys. Acta* 573(3), 443-50 (1979) Immunochemical methods for the selective measurement of pig post-heparin plasma lipoprotein lipase and hepatic lipase are described and validated. A simple two step purification method for porcine hepatic lipase from hepatic perfusate based on affinity chromatography and gel filtration is reported. The activity of the post-heparin plasma lipoprotein lipase and hepatic lipase in swine is reported. It is demonstrated that fasting decreases the activity of post-heparin plasma lipoprotein lipase activity more than two-fold while it does not affect the hepatic lipase activity significantly.

FACTORS AFFECTING THE ACTIVITY AND STABILITY OF THE PALMITOYL-COENZYME A HYDROLASE OF RAT BRAIN. T.E. Knauer (Dept. of Medicine, Med. College of Virginia, Richmond, VA 23298) *Biochem. J.* 179(3), 515-23 (1979). Palmitoyl-CoA hydrolase (EC 3.1.2.2) catalyses the irreversible hydrolysis of long-chain acyl-CoA thioesters. This enzyme is found primarily in the postmicrosomal supernatant fraction prepared from homogenates of rat brain. Either of two forms of the hydrolase, a lower-molecular-weight species of approx. 70,000 or a higher-molecular-weight species of approx. 130,000 can be isolated by gel filtration. The two forms differ in the availability or reactivity of certain external thiol groups, as determined by covalent chromatography with activated thiol Sepharose. The evidence supports the conclusion that the substrate palmitoyl-CoA promotes the formation of a relatively stable dimer from two unstable subunits. This process may not be reversible, since the removal of palmitoyl-CoA or glycerol from solutions of the higher-molecular-weight form does not result in the appearance of the lower-molecular-weight form of the hydrolase.

INCREASED CELLULAR PROLIFERATION IN ADIPOSE TISSUE OF ADULT RATS FED A HIGH-FAT DIET. B.J. Klyde and J. Hirsch (Rockefeller Univ., New York, NY 10021) *J. Lipid Res.* 20(6), 705-15 (1979). The feeding of a high-fat diet to adult rats was shown to increase the incorporation of [³H]thymidine into DNA of the adipocyte and stromal fractions. After only 2 days on a high-fat diet there was a marked increase in the incorporation of label. When a 2-week period was interposed between [³H]thymidine administration and determination of DNA specific activity, the greatest increase in incorporation of label was found after 1 week on the diet, when incorporation increased 6-fold or more in both adipocytes and stroma and subsequently decreased to stabilize at a level two or three times that of chow-fed rats in the adipocyte fraction. Rats labeled when young and later placed on a high-fat diet showed a decrease in DNA specific activity in both adipocytes and stroma, confirming that cellular proliferation had occurred in both fractions. The specific activities of both stromal and adipocyte DNA were very similar at all time points studied. An attempt to increase the difference in specific activities by waiting many weeks after [³H]thymidine injection before isolating DNA was not successful. This may be because the total amount of DNA in the stromal and adipocyte fractions increases in parallel on the diet. The significance of these findings in terms of the normal turnover of adipose tissue DNA and the responsiveness to diet is discussed.

RAT LIVER MICROSOMAL ELONGATION OF FATTY ACIDS. POSSIBLE INVOLVEMENT OF CYTOCHROME b₅. S.R. Keyes, J.A. Alfano, I. Jansson, and D.L. Cinti (Dept. of Pharmacology, Univ. of Connecticut Health Center, Farmington, CT 06032) *J. Biol. Chem.* 254(16), 7778-84 (1979). Participation of cytochrome b₅ in the liver microsomal elongation of fatty acids in the rat is suggested by (a) an increase in the rate of reoxidation of liver microsomal cytochrome b₅, following reduction by NADH, in the presence of both malonyl coenzyme A and ATP, and (b) a 60% inhibition of incorporation of malonyl-CoA into microsomal fatty acids in the presence of anti-cytochrome b₅ IgG; control IgG was without effect. A 2- to 4-fold increase in the rate of reoxidation of b₅ by malonyl-CoA was observed in liver microsomes from animals maintained on a fat-free diet, with maximal activity occurring after 48 h; a 1.4- to 1.5-fold increase was seen in control rats. Cyanide was found to inhibit the malonyl-CoA effect; however, the concentration of cyanide causing 50% inhibition was 1.25 mM; this was approximately 10 times greater than the concentration of cyanide causing 50% inhibition of the stearyl-CoA-enhanced rate. Lastly, the increase in the reoxidation rate by malonyl-CoA and ATP was not further enhanced by the addition of exogenous unsaturated and saturated fatty acids. The results are discussed in light of the two microsomal fatty acid biotransformation reactions, elongation and Δ⁹ desaturation.

INTERACTION OF BOVINE SERUM HIGH DENSITY LIPOPROTEIN WITH MIXED VESICLES OF PHOSPHATIDYLCHOLINE AND CHOLESTEROL. A. Jonas (Dept. of Biochem.,

School of Basic Med. Sciences and School of Chemical Sciences, Univ. of Illinois, Urbana, IL 61801) *J. Lipid Res.* 20(7), 817-24 (1979). The interaction of sonicated, small vesicles of egg phosphatidylcholine and cholesterol (2:1, mol/mol) with bovine high density serum lipoproteins was examined in terms of lipid transfer between both types of particles and the resulting changes in lipoprotein structure. Saturation of high density lipoprotein preparations with vesicle lipids gave final lipoprotein particles with essentially unchanged protein content and composition, unchanged cholesteryl ester and nonpolar lipid content, but with markedly increased phospholipid content (59% increase by weight) and moderately increased cholesterol content (20% increase by weight). The lipoproteins enriched in lipid were relatively uniform, spherical particles, $110 \pm 3.6 \text{ \AA}$ in diameter (6 \text{ \AA} larger than the original lipoproteins); they had a markedly decreased intrinsic protein fluorescence, a red-shifted fluorescence wavelength maximum, and more fluid lipid domains. These results indicate that the direct addition of excess lipids from membranes or other lipoproteins is a possible mechanism for lipid transfer to high density lipoproteins. Also they suggest a structural flexibility of high density lipoproteins that allows the addition of significant amounts of surface components.

EFFECT OF FEEDING THE SHELL FISH (*CORBICULA JAPONICA*) ON LIPID METABOLISM IN THE RAT. N. Iritani, E. Fukuda and K. Inoguchi (Tezukayama Gakuin College, Sumiyo-shiku, Osaka 558, Japan) *Atherosclerosis* 34(1), 41-7 (1979). Rats were maintained for 2 weeks on 3 different diets; a basal diet, one containing 0.1% cholate, and one containing 0.1% cholesterol and 0.1% cholate. Each dietary group was further divided into subgroups whose diet contained 0, 5, or 10% (dry weight) of minced *Corbicula japonica* (Prime). Feeding *Corbicula* significantly reduced the increase of cholesterol levels in rats fed the cholesterol diet. Though *Corbicula* contains several sterols, sterols other than cholesterol were almost not absorbed. Serum and liver triglyceride levels were significantly reduced by feeding *Corbicula* meat in all the dietary groups. Activities of glucose-6-phosphate dehydrogenase, malic enzyme and acetyl-CoA carboxylase were also markedly reduced by feeding *Corbicula*. The results suggest that *Corbicula* is a hypolipidemic food.

INTENSIFICATION OF ESSENTIAL FATTY ACID DEFICIENCY IN THE RAT BY DIETARY TRANS FATTY ACIDS. E.G. Hill, S.B. Johnson and R.T. Holman (The Hormel Inst., Univ. of Minn., Austin, MN 55912) *J. Nutr.* 109(10), 1759-65 (1979). Two studies were conducted using male rats to assess the effect of trans fatty acids upon essential fatty acid (EFA) deficiency. In the first study 5% corn oil (CO), hydrogenated coconut oil (HCNO) or margarine stock (MS, partially hydrogenated soybean oil) were fed, and the levels of trans fatty acids in tissue lipids were measured. The trans fatty acids present in MS were found to intensify EFA deficiency and to be retained in tissue lipids to a high degree, especially in heart phospholipids (PL). In the second study, as the level of trans fatty acids increased in the diet, increasingly higher levels of trans fatty acids were deposited in the heart PL. As dietary trans acids increased, a decrease in total $\omega 6$ fatty acids, and a decrease in the sum of $18:2\omega 6 + 20:4\omega 6 - 20:3\omega 9$ fatty acids in heart PL occurred, both criteria indicating a shift toward an increasing EFA deficiency state. Studies of $\Delta 5$ desaturase activity of liver microsomes in selected groups showed an increase in the conversion of $20:3\omega 6$ to $20:4\omega 6$ as the trans fatty acid level in the diet increased.

THE COMBINED EFFECT OF SMOKING AND COFFEE DRINKING ON LDL AND HDL CHOLESTEROL. S. Heyden, G. Heiss, C. Manegold, H.A. Tyroler, C.G. Hames, A.G. Bartel, and G. Cooper (Dept. of Community and Family Med., P.O. Box 2914, Duke Univ. Med. Center, Durham, NC 27710) *Circulation* 60(1), 22-5 (1979). Conflicting reports on the effect of smoking and coffee drinking on lipoproteins prompted us to study the combined effects of these two associated, widely prevalent habits in 361 persons randomly sampled from the Evans County cohort. Low-density lipoprotein (LDL) cholesterol levels were significantly higher among persons who smoked cigarettes and consumed five or more cups of coffee per day than among non-smokers who abstained from coffee. Conversely, high-density lipoprotein (HDL) cholesterol was higher in persons who did not smoke or drink coffee than in coffee-consuming smokers. However, this trend was not statistically significant. Triglycerides and very low-density lipoprotein (VLDL) cholesterol were highest among smokers who drank five or more cups of coffee per day, but these differences did not reach statistical significance. Lipoprotein cholesterol levels were adjusted for age, sex and body mass. Smoking and coffee drinking interact in affecting LDL and total cholesterol, but coffee drinking alone did not appear to affect blood lipids.

EFFECTS OF HIGH-GLUCOSE AND HIGH-FAT DIETS ON CONCAVALIN A BINDING TO RAT LIVER PLASMA MEM-

BRANES AND ON THE AMOUNT AND PATTERN OF THEIR GLYCOPROTEIN CARBOHYDRATES. D.S. Henriquez, H.M. Tepperman, and J. Tepperman (Dept. of Pharmacology, State Univ. of New York, Upstate Medical Center, Syracuse, NY 13210) *J. Lipid Res.* 20(5), 624-30 (1979). Purified liver plasma membranes were prepared from rats fed a high-fat, carbohydrate-free diet or a high-glucose, fat-free diet. Membranes from rats fed the high-fat diet bound significantly less ^{125}I -labeled concanavalin A (Con A) than did those from rats fed the fat-free diets. The magnitude of the binding difference increased with increasing concentrations of Con A. Neither association nor dissociation rates of the lectin-receptor complex was affected by diet. The extent of degradation of Con A by liver plasma membrane preparations from rats fed either diet was the same. Chemical analysis of delipidated liver plasma membrane showed that membranes prepared from high-fat diet-adapted rats had significantly lower values for all carbohydrate components measured with the exception of galactose. The results indicate that, in liver cells, a change in plasma membrane glycoproteins is part of the complex adaptation to altered diet composition.

TRIGLYCERIDE INTEGRATED CONCENTRATIONS: EFFECT OF VARIATION OF SOURCE AND AMOUNT OF DIETARY CARBOHYDRATE. J.T. Hayford, M.M. Danney, D. Wiebe, S. Roberts, R.G. Thompson (Dept. of Pediatrics, Univ. of Iowa College of Medicine, Iowa City, Iowa 52242) *Am. J. Clin. Nutr.* 32, 1670-8 (1979). The effect of changes in the amount of dietary carbohydrate (45 or 65% of total energy) and in the source of carbohydrate (sucrose or corn syrup) on plasma triglyceride and cholesterol concentrations was studied in eight healthy males. Subjects ingested each of four formula diets for 10 days in a latin square sequence. Diet-induced response was assessed by measurement of plasma lipid concentrations in blood obtained after overnight fast and by measurement of the mean plasma lipid concentrations - designated the integrated concentration - of blood obtained by 24 hr. continuous blood withdrawal. The fasting plasma triglyceride concentration increased significantly during ingestion of the high carbohydrate diet ($P < 0.005$) but was not significantly influenced by the source of the carbohydrate calories. The 45% carbohydrate diets induced larger meal associated plasma triglyceride variation than 65% diets. Sucrose-containing diets induced significantly higher plasma triglyceride integrated concentrations than corn syrup diets, whether provided as 45% ($P < 0.05$) or 65% ($P < 0.005$) of total energy. Diet-induced changes in fasting or integrated plasma cholesterol concentration were minimal.

UNUSUAL PROPERTIES OF RETINYL PALMITATE HYDROLASE ACTIVITY IN RAT LIVER. E.H. Harrison, J.E. Smith, and E.S. Goodman (Dept. of Medicine and Institute of Human Nutrition, Columbia Univ. College of Physicians and Surgeons, New York, NY 10032). *J. Lipid Research* 20(6), 760-71 (1979). These studies report the hydrolysis of retinyl palmitate with liver homogenates and homogenate fractions from retinol-depleted rats. The studies utilized an effective in vitro assay for retinyl palmitate hydrolase (RPH) activity, in which μg amounts of retinyl palmitate were employed as substrate, followed by the chromatographic separation and fluorescence assay of free and esterified retinol. The enzymatic activity showed an unusual subcellular distribution, with about 40% of total RPH activity recovered in the washed "nuclear" fraction (1,500 g pellet) and about 30-35% in the 105,000 g supernatant. RPH activity was not localized in any single, characterized subcellular structure. Another striking feature of the hepatic RPH activity was its extreme variability from rat to rat as assayed in vitro. Both the unusual subcellular distribution and the marked variability in activity were not observed for a variety of other hepatic ester hydrolase activities examined. The results suggest that the observed RPH activity is relatively specific for the hydrolysis of retinyl palmitate, and may therefore be significantly involved in hepatic retinyl ester metabolism.

ISOLATION AND INCORPORATION OF RABBIT LIVER EPOXIDE HYDRASE INTO PHOSPHOLIPID VESICLES. J. Halpert, H. Glaumann, and M. Ingelman-Sundberg (Dept. of Pharmacology, Karolinska Institute, S-104 01 Stockholm, Sweden) *J. Biol. Chem.* 254(15), 7434-41 (1979). Methods are described for the incorporation into phospholipid vesicles of epoxide hydrolase isolated from liver microsomes of phenobarbital-treated rabbits. At high substrate concentrations, the vesicles catalyzed the hydration of benzo(a)pyrene-4,5-oxide and styrene-7,8-epoxide at a rate similar to that obtained with the enzyme in a soluble form. However, the kinetics of styrene glycol formation catalyzed by the vesicular or microsomal preparations were complex. The results could be explained if reconstitution of the enzyme into the vesicles gives rise to low affinity high capacity sites for the substrate on the enzyme, or alternatively facilitates the interaction of the substrate with such sites already present. It is suggested that reconstituted liposomes containing both the liver microsomal hydroxylase system and epoxide hydrolase may prove to be a good model system for evalu-

ating substrate specificity and factors of importance in the formation of toxic and carcinogenic metabolites by these enzymes.

EFFECT OF GLUCOCORTICOIDS ON THE OXIDATIVE DESATURATION OF FATTY ACIDS BY RAT LIVER MICROSOMES. I.N.T. de Gómez Dumm, M.J.T. de Alaniz, and R.R. Brenner (Instituto de Fisiología, Facultad de Ciencias Médicas, Universidad Nacional de La Plata, Calle 60 y 120, 1900-La Plata, Argentina) *J. Lipid Res.* 20(7), 834-9 (1979). The effect of glucocorticoids on the oxidative desaturation of fatty acids by liver microsomal preparations of rats has been studied. Hydrocortisone produced a significant decrease in the conversion of [$1-^{14}C$] linoleic acid to γ -linolenic acid and [$1-^{14}C$] eicose-8,11,14-trienoic acid to arachidonic acid. Triamcinolone and dexamethasone were more active than hydrocortisone in depressing $\Delta 6$ and $\Delta 5$ fatty acid desaturating activity in liver microsomes. The glucocorticoids evoked a maximal response approximately 24 hr after admission. Palmitic acid conversion to palmitoleic acid showed no statistically significant changes by any of the glucocorticoids. The mechanism of action of glucocorticoids is apparently different from other hyperglycemic hormones that produce similar effects.

THE LOWERING OF PLASMA CHOLESTEROL BY SUCROSE POLYESTER IN SUBJECTS CONSUMING DIETS WITH 800, 300, OR LESS THAN 50 MG OF CHOLESTEROL PER DAY. C.J. Glueck, F.H. Mattson, R.J. Jandacek (Procter and Gamble Company, Miami Valley Laboratories, P.O. Box 39175, Cincinnati, Ohio 45247) *Am. J. Clin. Nutr.* 32(8), 1636-44 (1979). The efficacy, safety, acceptability, and cholesterol lowering potential of sucrose polyester (SPE), a liquid, nonabsorbable, fat-like material were assessed in normolipidemic men. Subsequently over three consecutive periods of 10 days each 8, 16, or 25 g of liquid SPE or 19, 38, or 62 g of an 80/20 mixture of SPE and completely hydrogenated palm oil (HPO) was added daily to the diets. Every treatment group but one showed at least a nominal decrease in plasma total and low density lipoprotein cholesterol. The responses of the plasma lipids are like those seen earlier with SPE that was a semisolid. Neither preparation resulted in any untoward effects, although the subjects preferred the mouth-feel of the liquid material that was used in this study. The vegetable oil-like culinary properties of liquid SPE should facilitate adherence to a regimen that includes this apparently safe and effective cholesterol-lowering agent.

CARNITINE PALMITOYLTRANSFERASE ACTIVITY IN MITOCHONDRIAL FRACTIONS ISOLATED FROM AORTAS OF RABBITS FED CHOLESTEROL-SUPPLEMENTED DIETS. P.J. Gillies and F.P. Bell (Diabetes and Atherosclerosis Research, The Upjohn Company, Kalamazoo, MI 49001) *Atherosclerosis* 34(1), 25-34 (1979). β -Oxidation of long-chain fatty acids increases manifold in atherosclerotic aortas; this may be due to an increase in the activity of the mitochondrial enzyme hexadecanoyl-CoA: carnitine O-hexadecanoyltransferase EC 2.3.1.23 (trivial name: carnitine palmitoyltransferase, CPT). To investigate this possibility, an assay for arterial CPT was developed and used to measure CPT activity in mitochondrial fractions isolated from aortas of rabbits fed high-fat (HF) or high-fat plus cholesterol (HFC) supplemented diets. It is concluded that the increase in β -oxidation of long-chain fatty acids in atherosclerosis is not attributable to an increase in CPT activity.

SIDE CHAIN CLEAVAGE OF SOME CHOLESTEROL ESTERS. F. Gasparini, A. Wolfson, R. Hochberg, and S. Lieberman (Depts. of Biochem. and of Obstetrics and Gynecology, and the International Inst. for the Study of Human Reproduction, The College of Physicians and Surgeons, Columbia Univ., New York, NY 10032) *J. Biol. Chem.* 254(14), 6650-6 (1979). For some time it has been known that the side chain of cholesterol sulfate is cleaved by the cleavage enzyme system present in bovine adrenal mitochondria without prior hydrolysis of the sulfate moiety. In this work, other inorganic esters as well as some organic esters of cholesterol were tested as substrates for this enzyme system. The results revealed that cholesterol nitrate, cholesterol phosphate, and a series of acyl esters of cholesterol can also be cleaved by the enzyme system to their respective pregnenolone derivatives without first being hydrolyzed to cholesterol. The rate of oxidation of the carboxylic acid esters decreased as the size of the acyl groups increased. Cholesterol stearate and cholesterol phosphate were demonstrated to be inhibitors of the side chain cleavage of cholesterol. While digitonin, as might be expected, inhibits the cleavage of cholesterol, it accelerates the oxidation of both cholesterol sulfate and cholesterol nitrate. The results reported in this paper add support to the previously proposed hypothesis that more than one cholesterol side chain cleavage enzyme system exists in adrenal mitochondria.

HETEROZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA. RELATIONSHIP BETWEEN PLASMA LIPIDS, LIPOPROTEINS, CLINICAL MANIFESTATIONS AND ISCHAEMIC HEART DISEASE IN MEN AND WOMEN. C. Gagné, S. Moorjani, D. Brun,

M. Toussaint and P.J. Lupien (Lipid Research Center, Laval Univ. Hospital, Quebec, P.Q. Canada) *Atherosclerosis* 34(1), 13-24 (1979). A large cohort of 264 men and 311 women with heterozygous familial hypercholesterolemia (FH) is analysed for the presence of xanthomas, ischaemic heart disease (IHD) and plasma lipids and lipoproteins. The plasma and low density lipoprotein (LDL) cholesterol are elevated to the same extent in both sexes, but on the contrary high density lipoprotein (HDL) cholesterol is decreased in both sexes as compared to normal controls. Thus an increase in LDL and a decrease in HDL may account for the early development of IHD in both men and women with FH. Although tendon xanthomas are equally observed in both sexes, IHD is not only precocious in men but its prevalence is also higher in men as compared to women. IHD is also more severe in men as seen from the higher incidence of myocardial infarction and fatal events. Irrespective of sex, the presence of tendinous xanthomas is related to elevated levels of both plasma and LDL cholesterol and higher LDL/HDL cholesterol ratio. The women with tendinous xanthomas, whether with or without IHD have similar levels of plasma lipids and lipoprotein cholesterol as compared to men with similar clinical manifestations. These findings lead us to suggest that lower prevalence of IHD in women with FH is due to their higher concentration of HDL-cholesterol.

ISOLATION AND CHARACTERIZATION OF 1 α -HYDROXY-23-CARBOXYTETRANORVITAMIN D: A MAJOR METABOLITE OF 1,25-DIHYDROXYVITAMIN D₃. R.P. Esvelt, H.K. Schooes, and H.F. DeLuca (Dept. of Biochemistry, College of Ag. and Life Sciences, Univ. of Wisconsin-Madison, Madison, WI 53706) *Biochemistry* 18(18), 3977-83 (1979). The *in vivo* side-chain oxidation of 1 α ,25-dihydroxyvitamin D₃ was investigated by using a double-label radiotracer technique. Rats dosed with 1 α ,25-dihydroxy [3α - 3H] vitamin D₃ and 1 α ,25-dihydroxy [$26,27$ - ^{14}C] vitamin D₃ produced compounds with a high $^3H/^{14}C$ ratio. These compounds were found in sizable quantities in intestine and liver within 3 h after dosing. The major side-chain oxidized metabolite migrated as an acid on DEAE-Sephadex chromatography and contained no ^{14}C . Methyl esterification of this compound with diazomethane proceeded in good yield and rendered the compound more amenable to chromatographic purification. The metabolite was isolated in several steps from rats dosed with 1 μ g of 1 α ,25-dihydroxy [3α - 3H] vitamin D₃. The metabolite was obtained in pure form as the methyl ester and was positively identified as 1 α ,3 β -dihydroxy-24-nor-9,10-seco-5,7,10(19)cholatrien-23-oic acid. The trivial name calcitric acid is proposed for this major side-chain oxidized metabolite of 1,25-dihydroxyvitamin D₃.

IN VITRO PRODUCTION OF HUMAN PLASMA LOW DENSITY LIPOPROTEIN-LIKE PARTICLES. A MODEL FOR VERY LOW DENSITY LIPOPROTEIN CATABOLISM. R.J. Deckelbaum, S. Eisenberg, M. Fainaru, Y. Barenholz and T. Olivecrona (Dept. of Pediatrics, Medicine B., and Biochem., Hadassah Univ. Hospital, Hebrew Univ.-Hadassah Med. School, Jerusalem, Israel) *J. Biol. Chem.* 254(13), 6079-87 (1979). To test whether human plasma low density lipoprotein (LDL) can be formed from very low density lipoprotein (VLDL) entirely in the plasma compartment, VLDL was incubated *in vitro* with purified bovine milk lipoprotein lipase and albumin. Analytical ultracentrifugation and electron microscopy show that *in vitro* "LDL" is less homogeneous and larger than native LDL removed from VLDL upon core triglyceride hydrolysis are present in the 1.019 to 1.063 g/ml density range, mainly as sac-like unilamellar liposomes, and in the 1.063 to 1.21 g/ml density range as discoidal particles. Therefore, lipolysis of VLDL using only an extrahepatic lipoprotein lipase can produce an apoprotein B-cholesterol ester-rich particle having many features in common with, but not identical to plasma LDL. Additional pathways must operate *in vivo* to form more homogeneous and smaller circulating LDL.

LYSOSOMAL TRIACYLGLYCEROL LIPASE AND LIPOLYSIS IN ISOLATED RAT HEPATOCYTES. L.J. Debeer, J. Thomas, P.J. De Schepper, and G.P. Mannaerts (Afdeling Farmakologie, Fakulteit Geneeskunde, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium) *J. Biol. Chem.* 254(18), 8841-6 (1979). Triacylglycerol lipase activity in homogenates from isolated rat hepatocytes was almost completely confined to the acidic region of the pH curve. The lysosomotropic agents chloroquine, ammonia, and methylamine inhibited endogenous fatty acid oxidation by 33 to 41% in isolated hepatocytes from fasted rats but did not interfere with the oxidation of exogenously added radioactive oleate. Endogenous fatty acid oxidation and proteolysis in isolated hepatocytes were inhibited to a similar extent by all three lysosomotropic agents suggesting a common mechanism for inhibition of lipolysis and proteolysis. Our data suggest that the acid lysosomal lipase is the only intracellular triacylglycerol lipase in the parenchymal cell and that the lysosomes are responsible for the breakdown of hepatic triglycerides.

ISOLATION, PROPERTIES, AND MECHANISM OF IN VITRO ACTION OF LECITHIN: CHOLESTEROL ACYLTRANSFERASE FROM HUMAN PLASMA. J. Chung, D.A. Abano, G.M. Fless, and A.M. Scanu (Depts. of Medicine and Biochemistry, Pritzker School of Medicine, and the Franklin McLean Memorial Research Institute, The Univ. of Chicago, Chicago, IL 60637) *J. Biol. Chem.* 254(15), 7456-64 (1979). A highly purified (about 17,000-fold) preparation of lecithin: cholesterol acyltransferase was obtained from fresh human plasma by a combination of ultracentrifugal and chromatographic techniques. These studies have shown that single bilayer vesicles are useful in the investigation of the mechanism of lecithin: cholesterol acyltransferase activity in vitro by providing a substrate of a precise surface geometry and composition. The studies also indicate that the difference in surface affinities between apo-A-I and apo-A-II plays a role in regulating the enzymatic activity in vitro. The relevance of these observations in vivo remains to be established.

THE STIMULATION OF ERUCATE METABOLISM IN ISOLATED RAT HEPATOCYTES BY RAPESEED OIL AND HYDROGENATED MARINE OIL-CONTAINING DIETS. R.Z. Christiansen, E.N. Christiansen and J. Bremer (Inst. of Medical Biochem., Sognsvannsveien 9, Oslo 3) *Biochim. Biophys. Acta* 573, 417-29 (1979). The metabolism of palmitate and especially of erucate was studied in hepatocytes isolated from rats fed for 3 weeks a diet containing peanut oil (diet 1), rapeseed oil (diet 2) and partially hydrogenated marine oil (diet 3). It is suggested that the increased metabolism of erucate in hepatocytes of marine oil and rapeseed oil-fed rats may be due to the increase in the peroxisomal β -oxidation.

METABOLISM OF ERUCIC ACID IN PERFUSED RAT LIVER: INCREASED CHAIN SHORTENING AFTER FEEDING PARTIALLY HYDROGENATED MARINE OIL AND RAPESEED OIL. E.N. Christiansen, M.S. Thomassen, R.Z. Christiansen, H. Osmundsen, and K.R. Norum (Institute for Nutrition Research, University of Oslo, Blindern, Oslo 3, Norway) *Lipids* 14(10), 829-35 (1979). The metabolism of [14 - 14 C] erucic acid was studied in perfused livers from rats fed on diets containing partially hydrogenated marine oil or rapeseed oil for three days or three weeks. Control rats were given groundnut oil. Chain-shortening of erucic acid, mainly to 18:1, was found in all dietary groups. In the marine oil and rapeseed oil groups, the percentage of chain-shortened fatty acids in very low density lipoproteins-triacylglycerols (VLDL-TG) exported from the liver increased after prolonged feeding. A similar increase was found in liver TG only with partially hydrogenated marine oil. This oil, rich in *trans* fatty acids, thus seemed to be more effective in promoting chain-shortening. The fatty acid composition of the secreted and stored TG differed both with respect to total fatty acids and radioactively labeled fatty acids, indicating that at least 2 different pools of TG exist in the liver. The lack of lipidosis in livers from rats fed dietary oils rich in 22:1 fatty acids is discussed in relation to these findings. In conclusion, a discussion is presented expressing the view that the reversal of the acute lipidosis in the hearts of rats fed rapeseed oil or partially hydrogenated marine oils is, to a large extent, derived from the increased chain-shortening capacity of erucic acid in liver.

CYTOCHROME C OXIDASE FROM BAKERS' YEAST: PHOTOLABELING OF SUBUNITS EXPOSED TO THE LIPID BILAYER. N. Cerletti and G. Schatz (Biocenter, Univ. of Basel, CH-4056 Bas 1, Switzerland) *J. Biol. Chem.* 254(16), 7746-51 (1979). Yeast mitochondria and purified yeast cytochrome *c* oxidase incorporated into micelles of the nonionic detergent Tween 80 were equilibrated with the hydrophobic aryl azides 5-[125 I]iodonaphthyl-1-azide or 5-(4-azido-2-nitrophenyl)-[35 S]thiophenol. The azides were then converted to highly reactive nitrenes by flash photolysis or by illumination for 2 min and the derivatized cytochrome *c* oxidase subunits were identified by gel electrophoresis and radioactivity measurements. 5-[125 I]iodonaphthyl-1-azide labeled mainly the three mitochondrially made Subunits I to III and the cytoplasmically made Subunit VII. Subunits IV to VI or cytochrome *c* bound to the purified enzyme were labeled 9- to 90-fold less. Essentially the same result was obtained with 5-(4-azido-2-nitrophenyl)-[35 S]thiophenol except that Subunit V was labeled as well. In contrast, all seven subunits as well as cytochrome *c* were heavily labeled when the enzyme was dissociated with dodecyl sulfate prior to photolabeling with either of the two probes. These data indicate that all subunits of yeast cytochrome *c* oxidase except Subunits IV and VI are at least partly embedded in the lipid bilayer of the mitochondrial inner membrane.

INSULIN INSENSITIVITY AND ALTERED GLUCOSE UTILIZATION IN CULTURED RAT ADIPOSE TISSUE. R.S. Bernstein (Medical Service, St. Luke's Hospital Center, New York, NY 10025) *J. Lipid Res.* 20(7), 848-56 (1979). Glucose utilization was studied in isolated fat cells prepared from rat adipose tissue which had been

cultured for 18 hr in TC 199 medium. There was no effect of insulin in the culture medium in any of the systems. Rates of free fatty acid and glycerol release were markedly increased in cultured cells, especially when insulin was present in the culture medium. The acute antilipolytic effect of insulin was retained, so that insulin in the test incubation decreased lipolysis by 40-80%. Nevertheless, cell-associated fatty acids were increased in cultured cells and FFA/albumin ratios in the medium often reached potentially toxic levels. The reduction in pentose phosphate shunt activity, lipogenesis, and insulin effect resembles other models of insulin insensitivity. The impaired metabolism is probably due to an intracellular defect. A possible toxic role of either intracellular or extracellular fatty acids cannot be excluded. This system should be a useful model in which to study the cellular mechanisms of insulin insensitivity in adipocytes.

METABOLISM OF ARACHIDONIC ACID IN POLYMORPHONUCLEAR LEUKOCYTES: STRUCTURAL ANALYSIS OF NOVEL HYDROXYLATED COMPOUNDS. P. Borgate and B. Samuelsson (Dept. of Chem., Karolinska Institutet, S-104 01 Stockholm, Sweden) *J. Biol. Chem.* 254(16), 7865-9 (1979). Arachidonic acid was incubated with rabbit peritoneal polymorphonuclear leukocytes (glycogen-induced) and compounds obtained from ether extractions were fractionated by silicic acid column chromatography. A fraction containing several unidentified metabolites of arachidonic acid was analyzed by reversed phase-high pressure liquid chromatography. The metabolites were esterified and further purified by silicic acid high pressure liquid chromatography. The structures of the pure compounds were elucidated by infrared and ultraviolet spectrometry, ozonolysis, and gas chromatography-mass spectrometry. The following novel compounds were identified: Compound 1, 5S, 12R-dihydroxy-(E,E,E,Z)-6,8,10,14-eicosatetraenoic acid; Compound 2, 5S, 12S-dihydroxy-(E,E,E,Z)-6,8,10,14-eicosatetraenoic acid; Compound 3, 5,6-dihydroxy-7,9,11,14-eicosatetraenoic acid; Compound 4, a diastereoisomer of the latter. Evidence for the occurrence of the δ -lactone forms of the 5,12-dihydroxy acids is also presented.

PANCREATIC COLIPASE: CHEMISTRY AND PHYSIOLOGY. B. Borgstrom, C. Erlanson-Albertsson, and T. Wieloch (Dept. of Physiological Chemistry, University of Lund, Lund, Sweden) *J. Lipid Res.* 20(7), 805-16 (1979). The following is an account of the present state of knowledge in this field which includes the chemistry, physical chemistry, and physiological chemistry of pancreatic colipase and its interactions.

DEGRADATION OF LOW DENSITY LIPOPROTEIN DEXTRAN SULFATE COMPLEXES ASSOCIATED WITH DEPOSITION OF CHOLESTERYL ESTERS IN MOUSE MACROPHAGES. S.K. Basu, M.S. Brown, Y.K. Ho, and J.L. Goldstein (Depts. of Molecular Genetics and Internal Medicine, Univ. of Texas Health Science Center at Dallas, Dallas, TX 75235) *J. Biol. Chem.* 254(15), 7141-6 (1979). The uptake and degradation of [125 I]-labeled human plasma low density lipoprotein (125 I-LDL) by mouse peritoneal macrophages was increased markedly by the addition of high molecular weight dextran sulfate ($M_r=500,000$) to the incubation medium. Competition studies with polyinosinic acid and fucoidin suggested that the LDL-dextran sulfate complex was recognized by a surface binding site that was different from the previously described site that mediates the uptake and degradation of acetylated LDL. The dextran sulfate-stimulated degradation of LDL enhanced the rate of cholesteryl ester formation in the macrophages, with a consequent marked increase in the cellular content of free and esterified cholesterol. These experiments indicate that macrophages have the capacity to ingest large amounts of LDL in association with high molecular weight-sulfated polysaccharides and that this ingestion leads to cholesteryl ester deposition in these cells.

ENZYME-SUBSTRATE INTERACTION IN LIPID MONOLAYERS. III. A STUDY OF THE VARIATION OF THE SURFACE CONCENTRATION WITH LIPOLYSIS. J.P. Barque and D.G. Dervichian (Dept. of Biophysics, Institut Pasteur, Paris 75015, France) *J. Lipid Res.* 20(5), 599-606 (1979). Monolayers of diacylglycerol were submitted to the action of lipase, keeping the area constant. The variation of the surface concentration Γ of the substrate with time was derived from the recorded reduction of the surface pressure π (the isotherm of the monolayer being previously established). The rate $-d\Gamma/dt$ was determined both as a function of the surface concentration Γ of the substrate and as a function of the bulk concentration C of the enzyme in the underlying solution. The rate depends on the quantity of enzyme z_e adsorbed on the monolayer and on the enzymatic specific activity a of these adsorbed enzyme molecules. Both z_e and a vary with Γ . The two variations have been quantitatively dissociated. The curves of z_e and of aa s functions of Γ coincide with those previously established in the study of hydrolysis under constant surface pressure.

SHORT-TERM EGG YOLK FEEDING IN HUMANS. INCREASE IN APOLIPOPROTEIN B AND LOW DENSITY LIPOPROTEIN CHOLESTEROL. D. Applebaum-Bowden, W.R. Hazzard, J. Cain, M.C. Cheung, R.S. Kushwaha and J.J. Albers (Howard Hughes Med. Institute Labs. and the Northwest Lipid Research Clinic, Univ. of Washington, School of Med., Seattle, WA 98195) *Atherosclerosis* 33(4): 385-96 (1979). In animal studies, hypercholesterolemia induced by cholesterol feeding results in the plasma cholesterol being transported by lipoproteins of lower densities. Little information is available for humans. To determine the specific lipoprotein responses to dietary cholesterol challenge in humans, four volunteer subjects ingested a liquid formula diet containing 5000 mg of egg yolk cholesterol per day for 30 days and the changes in their lipoprotein fractions were examined. The high dietary cholesterol (above the range of normal diet) was associated with marked increases in apolipoprotein B and low density lipoprotein (LDL) cholesterol levels. An elevated cholesterol:triglyceride ratio in the LDL fraction indicated that the diet altered both LDL level and composition. High density lipoprotein cholesterol and apolipoprotein AI increased slightly. Very low and intermediate density lipoprotein cholesterol and apolipoprotein E levels did not increase during the diet. Thus, high dietary cholesterol was associated with major changes in LDL level and composition, but only minor changes in the other lipoprotein fractions and suggested only minor accumulation of remnant particles.

PHOSPHOLIPID ACTIVATION OF COBRA VENOM PHOSPHOLIPASE A₂. 1. LIPID-LIPID OR LIPID-ENZYME INTERACTION. M.F. Roberts, M. Adamich, R.J. Robson and E.A. Dennis (Dept. of Chem., Univ. of Calif. at San Diego, La Jolla, Calif.) *Biochemistry* 18(15), 3301-8 (1979). In individual phospholipid-Triton X-100 mixed micelles, phospholipase A₂ hydrolyzes phosphatidylcholine at a tenfold higher rate than phosphatidylethanolamine, while in binary phospholipid mixtures phosphatidylcholine activates the enzyme toward phosphatidylethanolamine so that it becomes the preferred substrate. This specificity reversal has now been observed in anionic and zwitterionic as well as in nonionic detergent mixed micelle systems. Detailed studies on the physical characteristics of Triton X-100/phospholipid mixed micelles were conducted to ascertain their role in the activation process.

MEASUREMENTS OF FATTY ACID SYNTHESIS BY INCORPORATION OF DEUTERIUM FROM DEUTERATED WATER. G.M. Patton and J.M. Lowenstein (Grad. Dept. of Biochem., Brandeis Univ., Waltham, MA 02154) *Biochemistry* 18(14), 3186-88 (1979). Fatty acid synthesis by perfused rat livers is measured by using D₂O as tracer. The newly synthesized, deuterium-labeled fatty acids are separated from unlabeled fatty acids by gas chromatography using glass capillary columns. The areas of the deuterium-labeled peaks are proportional to the amounts of fatty acids synthesized. The absolute amounts of the individual fatty acids synthesized are obtained by use of an internal standard. The number of deuterium atoms incorporated, as determined by mass spectrometry, is proportional to the D₂O concentration of the perfusate, except at very high concentrations of D₂O. The relative retention times of the newly synthesized, deuterium-labeled fatty acids are proportional to their deuterium content.

INHIBITORS OF STEROL BIOSYNTHESIS. SYNTHESIS OF 14 α -ALKYL SUBSTITUTED 15-OXYGENATED STEROLS. E.J. Parish, M. Tsuda and G.J. Schroepfer, Jr. (Depts. of Biochem. and Chem., Rice Univ., Houston, TX 77001) *Chem. Phys. Lipids* 24(3), 209-36 (1979). The chemical syntheses of a number of 14 α -alkyl substituted 15-oxygenated sterols have been pursued to permit evaluation of their activity in the inhibition of the biosynthesis of cholesterol and other biological effects. Described herein are the first chemical syntheses of oxygenated sterols.

TIME-DEPENDENT CHANGES IN THE SIZE DISTRIBUTION OF DISTEAROYLPHOSPHATIDYLCHOLINE VESICLES. A.L. Larrabee (The Procter & Gamble Co., Miami Valley Laboratories, Cincinnati, OH 45247) *Biochemistry* 18(15), 3321-6 (1979). The results of transmission electron microscopic and ultracentrifugal studies of the size distribution of sonicated distearoylphosphatidylcholine vesicles are reported. Small vesicles ($d \approx 300$ Å) were prepared by sonication of pure 1,2-distearoyl-3-*sn*-phosphatidylcholine in water and incubated at 4, 21, 40, 53, and 65°C. The vesicle size distributions changed as a function of time at all temperatures below the phase-transition temperature but remained constant at the transition temperature and above.

MEDIUM-CHAIN FATTY ACID SYNTHESIS IN LACTATING-RABBIT MAMMARY GLAND: INTRACELLULAR CONCENTRATION AND SPECIFICITY OF MEDIUM-CHAIN ACYL THIOESTER HYDROLASE. J. Knudsen (Inst. of Biochem., Odense Univ., 5230-Odense M, Denmark) *Biochem. J.* 181(2), 267-74 (1979). The concentration of medium-chain acyl thioester hydrolase

and of fatty acid synthetase was determined by rocket immunoelectrophoresis in nine different particle-free supernatant fractions from lactating-rabbit mammary gland. The molar ratio of the hydrolase to fatty acid synthetase was 1.99 ± 0.66 (mean \pm S.D.). A rate-limiting concentration of malonyl-CoA was required to ensure the predominant synthesis of medium-chain fatty acids when 2 mol of the hydrolase was added per mol of fatty acid synthetase. The interaction of the hydrolase with fatty acid synthetase was concentration-dependent, though an optimum concentration of hydrolase to synthetase could not be obtained. The lactating-rabbit mammary gland hydrolase altered the pattern of fatty acids synthesized by fatty acid synthetases prepared from cow, goat, sheep and rabbit lactating mammary glands, rabbit liver and cow adipose tissue.

ACTION OF COBRA VENOM PHOSPHOLIPASE A₂ ON THE GEL AND LIQUID CRYSTALLINE STATES OF DIMYRISTOYL AND DIPALMITOYL PHOSPHATIDYLCHOLINE VESICLES. C.R. Kensil and E.A. Dennis (Dept. of Chem., Univ. of Calif. at San Diego, La Jolla, CA 92093) *J. Biol. Chem.* 254(13), 5843-8 (1979). The activity of phospholipase A₂ from cobra venom toward phospholipid in single-walled, sonicated vesicles was analyzed, particularly with respect to its activity toward the saturated phosphatidylcholines in the gel and liquid crystalline states. The results suggest that, toward sonicated vesicles, there is no specific enhancement of the rate when both liquid crystalline and gel states are present together, as has been suggested to occur for multibilayers studied with other phospholipases. An apparent stimulation of activity as the reaction proceeded was observed above the phase transition temperature. This might be attributed to an increase in the phase transition temperature caused by free fatty acids so that, in the presence of reaction products, the enzyme is actually hydrolyzing gel state phospholipid which was found to be the preferred lipid state for phospholipase activity.

THERMAL STABILITY OF FATTY ACID-SERUM ALBUMIN COMPLEXES STUDIED BY DIFFERENTIAL SCANNING CALORIMETRY. S. Gumpen, P.O. Hegg and H. Martens (Norwegian Food Research Institute, P.O. Box 50, N-1432 Aas-NLH Norway) *Biochim. Biophys. Acta* 574(2), 189-96 (1979). Differential scanning calorimetry has been used to study the thermal stability of bovine serum albumin as affected by binding of fatty acids (lauric acid and stearic acid) and sodium dodecyl sulfate (SDS). All the ligands stabilized the protein molecules in a similar manner, but to different levels. A maximum increase in denaturation temperature of 30°C was obtained with lauric acid. The thermograms indicate the presence of several ligand-albumin complexes having different heat stabilities. Variations in pH in 0.9% NaCl affected the heat stability of both ligand-poor and ligand-rich albumin, the former being more sensitive to variations in pH within the physiological range. Variations in NaCl concentration affected the thermal stabilities at neutral pH, especially at low salt concentrations. While ligand-rich albumin was somewhat destabilized by increasing NaCl concentrations, ligand-poor albumin was strongly stabilized. The potential use of differential scanning calorimetry in ligand-albumin research is discussed.

CARBON-13 NUCLEAR MAGNETIC RESONANCE STUDIES OF THE INTERACTION OF LECITHIN WITH PURIFIED D-HYDROXYBUTYRATE APODEHYDROGENASE, A LIPID-REQUIRING ENZYME. S. Fleischer, J.O. McIntyre, W. Stoffel, and B.D. Tunggal (Dept. of Molecular Bio., Vanderbilt Univ., Nashville, TN 37235) *Biochemistry* 18(11), 2420-9 (1979). The interaction of D- β -hydroxybutyrate apodehydrogenase (BDH), a lecithin-requiring enzyme purified from bovine heart mitochondria, with lecithin (PC) has been studied by ¹³C NMR by using lecithin labeled either in the polar moiety, soybean [N -¹³CH₃]PC, or in the hydrophobic moiety, [11 -¹³C]dioleoyl-PC. These studies show that, upon interaction of BDH with phospholipid vesicles containing lecithin, the rotational motion in the polar group of lecithin is constrained, whereas the motion in the hydrophobic region is increased. The increased motion of the hydrophobic moiety could result from disorder in the bilayer. Further, the line shape of the [11 -¹³C]lecithin resonance was broadened upon interaction with BDH. Such line broadening could result from chemical shift anisotropy or constrained lateral motion. The interaction of BDH with lecithin in phospholipid vesicles, as measured by ¹³C NMR, is unique as compared with the two other systems previously studied.

Fats and oils

DETECTION OF SHEA BUTTER IN COCOA BUTTER. M. Derbesy and M.T. Richert, *Oleagineux*, 34, 405-9 (1979). The unsaponifiable part of shea butter contains certain specific constituents which, when found, enable the detection of this butter, even in very small quantities, in confectionery fats, in particular

when mixtures of shea butter and cocoa butter are concerned. Use of modern analysis methods for rapid detection, applicable to industrial checks as well as to the quantitative determination of shea butter in cocoa-shea mixtures.

USE OF BIOCHEMICAL METHODS FOR FAT AND OIL ANALYSIS. APPLICATION OF THE DETERMINATION OF CHOLESTEROL. S. Chemin Douaud and A. Karleskind. *Rev. Fr. Corps Gras*, 26, 313-6 (1979). This method uses several enzymes: the oxidase cholesterol and the catalase. During the reactions, a colored compound called lutidine is produced. The color intensity measured at 450 nm is proportional to content of cholesterol. This analysis is fast and accurate.

DETERMINATION OF PHOSPHORUS IN VEGETABLE OILS BY FLAMELESS ATOMIC ABSORPTION. INFLUENCE OF LANTHANUM. M. Gente-Jauniaux and A. Prevot. *Rev. Fr. Corps Gras*, 26, 325-9 (1979). The determination of phosphorus in vegetable oils by flameless atomic absorption with addition of lanthanum as cyclohexanebutyrate is able, on the one hand, to suppress the differences of responses obtained without lanthanum for some samples, on the other hand, to increase distinctly the sensitivity of the method. The validity of this method has been verified by means of lecithins used to draw a standard curve.

FORMATION OF UNSATURATED FATTY ACIDS IN LINSEED (*Linum usitatissimum* L.). A. Cherif and P. Mazliak. *Oleagineux* 34, 301-7 (1979). The accumulation of fats was followed throughout the formation and ripening of linseed. Three phases were observed: a phase of slow accumulation, another of rapid accumulation and a third of either slow or nil accumulation. As the linseed develops, the fatty acids undergo variations, and linolenic becomes the characteristic fatty acid of linseed oil. The desaturating activity of ammonium oleate is important in the flax flower and in the first stages of development of the seed.

PHENOLIC MATERIALS OF SUNFLOWER SEEDS DURING THE MATURATION PROCESS. O.P. Mironova et al. *Pishch. Tekhnol.* 1979(2), 11-3. (Rev. Fr. Corps Gras).

STUDY BY THE EXPERIMENTAL - STATISTICAL METHODS OF THE VARIATIONS OF THE ACID VALUE OF SUNFLOWER OIL DURING EXTRACTION. A.M. Malyshev et al. *Pishch. Tekhnol.* 1979(3), 67-9. The obtained regression equation reflects, in an adequate manner, the influence of a series of technological factors and of the quality of the raw material on the variation of the acid value of the oil during extraction. The factors which influence the acid value can be classified in the following order: passing of the flake through a sieve of 1 mm; acid value of the oil in the seed at the start; ratio solvent:material; temperature of the micelle. (Rev. Fr. Corps Gras).

COMPOSITION OF WAX ESTERS AND TRIACYLGLYCEROLS IN THE MELON AND BLUBBER FATS OF A YOUNG SOWERBY'S WHALE *MESOPLODON BIDENS*. C.M. Lok and B. Folkersma (Unilever Research, Vlaardingen, The Netherlands) *Lipids* 14(10), 872-5 (1979). The blubber fat of a yearling Sowerby's Whale, *Mesoplodon bidens*, stranded on the Dutch coast, contained 59% triacylglycerols. This is quite unexpected because low levels (0-6%) of triacylglycerols are characteristic of *Ziphiidae* whales. In addition, the chain lengths of the fatty acids of the melon were longer than those of previously studied related species. As young Sowerby's Whales undergo a change in diet from mainly triacylglycerols in milk to wax ester containing food during their development from infancy to independence, these findings could reflect the age of the animal.

STRUCTURAL AND KINETIC STUDIES ON THE SOLUBILIZATION OF LECITHIN BY SODIUM DEOXYCHOLATE. D. Lichtenberg, Y. Zilberman, P. Greenzaid, and S. Zamir (Depts. of Pharmacology, Biochemistry, and Statistics, The Hebrew Univ., Jerusalem, Israel) *Biochemistry* 18(16), 3517-25 (1979). Mixed dispersions of egg phosphatidylcholine (PC) and the bile salt sodium deoxycholate (DOC) were prepared by various methods, and their turbidities and proton magnetic resonance spectra were studied as a function of time. The spectra of dispersions prepared by dissolving both components in a common organic solvent and replacing the organic solvent by water did not change with time, indicating that the mixed aggregates formed represent "a state of equilibrium". Upon mixing PC with aqueous solutions of DOC, we found that the mixed aggregates formed are slowly reorganized and ultimately reach the same state of equilibrium. This reorganization was found to be a pseudo-first-order process, the rate constant of which depends linearly upon the detergent concentration. This process involves saturation of the outer bilayers of the multi-lamellar PC by detergent, followed by transformation of these bilayers into mixed micelles. It is concluded that the solubilization occurs through consecutive "peeling off" of lecithin bilayers.

SYNTHESIS AND ENZYMATIC CONVERSION OF AN ETHER ANALOGUE OF MONOGLACTOSYL DIACYLGLYCEROL. E. Heinz, H.P. Sibertz and M. Linscheid (Institutes of Botany and Organic Chem., Univ. of Cologne, D-5000 K8ln 41, Gyrhofstr. 15 and Greinstr. 4, F.R.G.) *Chem. Phys. Lipids* 24(3), 265-76 (1979). The synthesis of 1,2-di-O-9'-octadecenyl-3-O-β-D-galactopyranosyl-sn-glycerol is described, including a detailed discussion of mass spectrometric fragmentation patterns of this and related substances. Enzymatic experiments showed that this compound is converted by plant enzymes to the 6-O-acyl derivative. The availability of the di-9-octadecenyl compound for tritium reduction will provide a substrate for studies on direct desaturation of lipid-linked acyl or alkyl chains.

MEASUREMENT OF ARACHIDONIC ACID IN THE PLASMA BY GAS-LIQUID CHROMATOGRAPHY-FLAME IONIZATION USING DIHOMO-γ-LINOLENIC ACID AS AN INTERNAL STANDARD. J.G. Gerber, J.S. Barnes, and A.S. Nies (Univ. of Colorado Medical Center, Div. of Clinical Pharmacology, Denver, CO 80262) *J. Lipid Res.* 20(7), 912-8 (1979). A gas-liquid chromatography-flame ionization method is described for measuring arachidonic acid in plasma using dihomo-γ-linolenic acid as an internal standard. We found this technique to be reproducible, and quicker and superior to previously described techniques because of the similar physico-chemical properties of the unsaturated fatty acid internal standard and arachidonic acid. The use of the saturated fatty acid, n-tricosanoic acid, was unsatisfactory as an internal standard because of its poor extractability from plasma as compared to arachidonic acid.

THE MECHANISM OF NADPH-DEPENDENT LIPID PEROXIDATION: THE PROPAGATION OF LIPID PEROXIDATION. B.A. Svingen, J.A. Buege, F.O. O'Neal, and S.D. Aust (Dept. of Biochem., Michigan State Univ., East Lansing, MI 48824) *J. Biol. Chem.* 254(13), 5892-9 (1979). NADPH-dependent lipid peroxidation occurs in two distinct sequential radical steps. The first step, initiation, is the ADP-perferryl ion-catalyzed formation of low levels of lipid hydroperoxides. The second step, propagation, is the iron-catalyzed breakdown of lipid hydroperoxides formed during initiation generating reactive intermediates and products characteristic of lipid peroxidation. Propagation results in the rapid formation of thiobarbituric acid-reactive material and lipid hydroperoxides. Propagation can be catalyzed by ethylenediamine tetraacetate-chelated ferrous ion, diethylene triamine pentaacetic acid-chelated ferrous ion, or by ferric cytochrome P-450. However, cytochrome P-450 is destroyed during propagation.

EFFECTS OF PHOSPHOLIPID ACYL CHAIN STRUCTURE ON PHYSICAL PROPERTIES: I. ISOBRANCHED PHOSPHATIDYLCHOLINES. J.R. Silvius and R.N. McElhaney (Dept. of Biochem., Univ. of Alberta, Edmonton, Alberta T6G 2H7, Canada) *Chem. Phys. Lipids* 24(3), 287-96 (1979). All of the isobranched fatty acids of 12 to 18 carbons have been synthesized in gram quantities by a convenient acetylene coupling reaction followed by catalytic hydrogenation. The corresponding phosphatidylcholines (PCs) have been synthesized and their thermotropic phase behavior investigated by differential thermal analysis. The isobranched acyl phosphatidylcholines show gel-to-liquid-crystalline phase transition temperatures (T_c) some 20° C below those of the corresponding straight-chain PCs and appear to exhibit two slowly interconverting low-temperature phases below T_c . The observed strong alternation of T_c s between isobranched PCs with odd- and even-carbon number acyl chains contrasts with the behavior of the straight-chain PCs and suggests that the acyl chains of the branched-chain PCs are strongly tilted with respect to the bilayer normal below and/or above T_c while those of the straight-chain PCs are not. These results clearly indicate significant differences in the overall packing of branched- and straight-chain PCs in the gel and possibly the liquid-crystalline state.

CHANGES IN THE ACYL LIPID COMPOSITION OF PHOTOSYNTHETIC BACTERIA GROWN UNDER PHOTOSYNTHETIC AND NON-PHOTOSYNTHETIC CONDITIONS. N.J. Russell and J.L. Harwood (Dept. of Biochem., Univ. College, P.O. Box 78, Cardiff CF1 1XL, Wales, United Kingdom) *Biochem. J.* 181(2):339-45 (1979). The acyl lipids and their constituent fatty acids were studied in the photosynthetic bacteria *Rhodospirillum rubrum*, *Rhodopseudomonas capsulata* and *Rhodopseudomonas sphaeroides*. The major lipids were found to be phosphatidylethanolamine, phosphatidylglycerol and cardiolipin in each bacterium. The two *Rhodopseudomonas* species also contained significant quantities of phosphatidylcholine. Analysis of the lipids of chromatophores, isolated from the three bacteria, showed that these preparations were enriched in phosphatidylglycerol. The large increase in the phospholipid, seen during growth under photosynthetic conditions, appeared, therefore, to be due to a proliferation of chromatophore membranes. Possible roles for acyl lipids in the forma-

tion and function of the photosynthetic apparatus of bacteria are discussed.

MEMBRANE LIPIDS OF MYCOPLASMA GALLISEPTICUM: A DISATURATED PHOSPHATIDYLCHOLINE AND A PHOSPHATIDYLGLYCEROL WITH AN UNUSUAL POSITIONAL DISTRIBUTION OF FATTY ACIDS. S. Rottem and O. Markowitz (Biomembrane Res. Lab., Dept. of Clinical Microbio., The Hebrew Univ.-Hadassah Med. School, Jerusalem, Israel) *Biochemistry* 18(14), 2930-5 (1979). The lipid content of *Mycoplasma gallisepticum* depended on the growth phase of the culture, being high in cells harvested at the early logarithmic phase of growth and low in stationary phase cells. The phospholipid fraction is comprised of three major compounds, tentatively identified as sphingomyelin (SPM), phosphatidylcholine (PC), and phosphatidylglycerol (PG). When grown with increasing serum concentrations, the relative content of PG decreased while that of PC and SPM increased. PG is the only phospholipid de novo synthesized by the organisms. It has an unusual positional distribution of fatty acids. Fatty acids with lower melting points are located primarily at position 1 and fatty acids with higher melting points at position 2 of the *sn*-glycerol 3-phosphate.

CHEMICAL SYNTHESIS OF THREE 14 α -HYDROXYMETHYLCHOLESTENOLS. R.A. Pascal, Jr., R. Shaw, and G.J. Schroeffer,

Jr. (Depts. of Biochem. and Chem., Rice Univ., Houston, TX 77001) *J. Lipid Res.* 20(5), 570-8 (1979). Reported herein are chemical syntheses of 14 α -hydroxymethyl-5 α -cholest-8-en-3 β -ol, 14 α -hydroxymethyl-5 α -cholest-7-en-3 β -ol, and 14 α -hydroxymethyl-5 α -cholest-6-en-3 β -ol. These compounds were obtained in pure form after repeated medium-pressure column chromatography of the mixture obtained by treatment of 3 β -acetoxy-7 α , 32-epoxy-14 α -methyl-5 α -cholestane with pyridine hydrochloride in refluxing acetic anhydride followed by reduction with lithium aluminum hydride. The compounds were characterized by their chromatographic properties and by the results of infrared, optical rotation, nuclear magnetic resonance, and low and high resolution mass spectral studies.

LIPID COMPOSITION OF DARK AND WHITE MUSCLE FROM WHITE SUCKER (*CATOSTOMUS COMMERSONI*). J. Mai and J.E. Kinsella (Dept. of Food Science, Cornell Univ., Ithaca, NY 14853) *J. Food Sci.* 44(4), 1101-5 (1979). The lipids from dark and white muscle of white sucker (*Catostomus commersoni*) were fractionated by thin-layer chromatography and fatty acid composition of each lipid class studied by gas-liquid chromatography. Dark muscle has a higher content of total lipid than white muscle (6.2% vs 1.4%) which was mostly accounted for by triglycerides. Phospholipid (PL) concentration was 521 \pm 47 and 395 \pm 34 mg/100 of dark and white muscle, respectively. Phosphatidylcholine was the predominant PL class in both muscles though the concentrations of other PL classes were much higher in the dark muscle. Palmitic acid (16:0), palmitoleic acid (16:1 n7), oleic acid (18:1 n9), eicosapentaenoic acid (20:5 n3) and docosahexaenoic acid (22:6 n3) were the major fatty acids present. White muscle contained higher percentages of polyunsaturated fatty acid (PUFA) than the dark muscle. The free fatty acid concentration was about the same (approx. 7%) in both dark and white muscle.

THE SOLUBILISATION OF SOME STEROIDS BY PHOSPHATIDYLCHOLINE AND PHOSPHATIDYLCHOLINE-CHOLESTEROL VESICLES. B. Lundberg (Dept. of Biochem. and Pharmacy, Abo Akademi, SF-20500 Abo, Finland) *Chem. Phys. Lipids* 24(3), 257-63 (1979). The solubility of the three steroid hormones, progesterone, testosterone, and estradiol-17 β in water and phosphatidylcholine vesicles was measured after shaking and ultrasonication. All three steroids have low water solubility, which increases considerably at sonication for testosterone and estradiol-17 β . The phosphatidylcholine vesicles have a very small solubilising capacity for the steroids; about 20 μ mol/mol. This increases at soni-

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cation for estradiol-17 β and decreases for testosterone. The capacity for progesterone is almost unaltered. The incorporation of cholesterol in the vesicles decreased the solubilisation capacity for testosterone and estradiol-17 β but increased that for progesterone of shaken preparations. For the sonicated systems the cholesterol decreased the solubilising capacity for estradiol-17 β but increased that for testosterone. The solubilisation experiments indicate that the steroid hormones are solubilised in the hydrocarbon part of the phosphatidylcholine bilayer and also ¹³C NMR results support this conclusion.

A HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF TOCOPHEROL IN PLASMA AND CELLULAR ELEMENTS OF THE BLOOD. L.J. Hatam and H.J. Kayden (Dept. of Med., New York Univ. Med. Center, New York NY 10016) *J. Lipid Res.* 20(5), 639-45 (1979). A rapid, sensitive, and reproducible procedure is described for the analysis of α -tocopherol in blood cells and plasma using high-performance liquid chromatography and fluorometric detection. The cardinal feature for the increased sensitivity of this high-performance liquid chromatographic procedure is that the fluorometric analysis was carried out at a short excitation wavelength (205 nm) which increased the sensitivity 20 fold over the usual excitation wavelength of 295 nm. Tocopherol levels can be measured in as little as 50 μ l of plasma and 200 μ l of erythrocytes. The tocopherol content of plasma, red blood cells, platelets, polymorphonuclear leukocytes, and lymphocytes of normal subjects and subjects ingesting additional quantities vitamin E are reported. The values for the white cells are approximately 30 times higher than those of the red blood cells (polymorphonuclear leukocytes $4.47 \pm 0.62 \mu\text{g}/10^9$, lymphocytes $3.89 \pm 0.85 \mu\text{g}/10^9$, and erythrocytes $1.40 \pm 0.17 \mu\text{g}/10^{10}$ cells). The tocopherol contents of the plasma and all the cellular elements of the blood were increased by oral feeding with vitamin E.

PUBLICATIONS ABSTRACTED

- American Journal of Clinical Nutrition, 9650 Rockville Pike, Bethesda, MD 20014.
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- Journal of Lipid Research, F.A.S.E.B. (Federation of American Societies for Experimental Biology), 9650 Rockville Pike, Bethesda, MD 20014.

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- Journal of Oil & Colour Chemists' Association, Priory House, 967 Harrow Road, Wembley HAO 2SF Middlesex, England.
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- Journal of Food Science, Institute of Food Technology, Suite 2120, 220 N. LaSalle St., Chicago, IL 60601.
- Journal of the Society of Cosmetic Chemists, 1905 Broadway, Suite 1701, New York, NY 10023.
- Lipids, American Oil Chemists' Society, 508 S. Sixth St., Champaign, IL 61820.
- Paint Research Association, Waldegrave Road, Teddington, Middlesex TW11-8LD, Great Britain.
- Paintindia, Color Publications Pvt. Ltd., 126-A Dhuruwadi, Prabhadevi, Bombay 400 025, India.
- Poultry Science, 309 W. Clark St., Champaign, IL 61820.
- Proceedings of the Society of Experimental Biology and Medicine, 630 W. 168th St., New York, NY 10032.
- Science, American Association for the Advancement of Science, 1515 Massachusetts Avenue, Washington, DC 20005.
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